REMARKS

Claims 1–4, 10, 15–20, 22–28, 30–37, 39–43, 61–63, 67–70, and 79–82 are currently pending with claims 11–13, 29, 38, 44–60, 64–66, 71, and 74–78 withdrawn from consideration. Claims 5–9, 14, 21, and 72–73 are canceled without prejudice or disclaimer.

Claim Objections

The Examiner's careful reading of the claims is appreciated. The minor informality in claim 81 has been corrected according to the Examiner's suggestion.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1–4, 8, 10, 15–20, 22–28, 30–37, 39–43, 61–63, 67–70, and 79–82 stand rejected under 35 U.S.C. § 112, first paragraph for allegedly being non-enabled. Applicants respectfully traverse this rejection.

Applicants appreciate the examiner's statement on the record with respect to the conventionality of information necessary to construct fusion proteins. The examiner's position now seems to be based on an allegation that the reference to amino acid sequences in the claims permits retention of as little as two consecutive amino acids from reference sequences. This, it is respectfully submitted, constitutes a misreading of the claims. At all times, and hopefully now even more clearly with the foregoing editorial changes, the amino acid sequence referred to in the claim must be one which retains the specific functions recited for such sequences in the claims. Consequently, the alleged unlimited variability which underlies the examiner's problem with the claims in fact does not exist. Thus, the written description rejection must be withdrawn.

The same conclusion applies to the enablement rejection because the examiner seems to be relying on the same sort of interpretation of the claims as support for an allegation of a greatly expanded scope.

As can be seen, the subject matter of the claims can be made and used with, at most, routine experimentation using conventional component parts.

Claim 43 depends on claim 36. Consequently, the nuclear receptor mentioned in the claim 43, by virtue of the recitations present in claim 36, is a steroid receptor. The redundancy in claim 63 has been removed.

Rejections Under 35 U.S.C. § 112, second paragraph

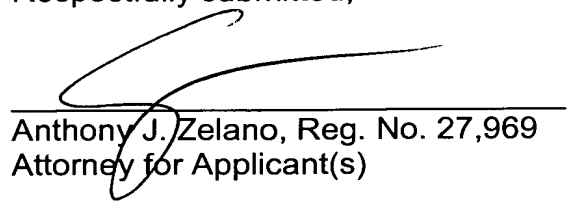
Claims 8, 10, and 81 stand rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. Applicants respectfully traverse these rejections.

In item 11 of the open Office Action, it is alleged that the claimed fusion protein comprising a constitutively active Ras protein in third domain is contradictory to claim 1, which *seems* to describe the third domain as being regulated by ligand binding to the second domain. Applicants respectfully disagree. Applicants submit that the specified activity according to claim 10 is also the activity of the Ras protein, which is only activated when the ligand binds to the second domain of the fusion protein according to claim 1 of the instant invention. According to claim 10, this activity of the Ras protein is specified to be constitutive. See, page 21, lines 17–30 of the instant specification. However, in order to facilitate prosecution, Applicants have amended claim 10 to recite this limitation. This "constitutive" aspect is a conventional phenomenon. See the diverse abstracts being filed herewith.

The rejection of claim 8 is moot in view of its cancellation. Additionally, the rejection of claim 81 is rendered moot in view of its amendment. Therefore, it is respectfully requested that the rejections be withdrawn.

A check in the amount of $450.00 is enclosed for the two-month extension of time feel. No other fees are believed to be due with this response; however, the Commissioner is hereby authorized to charge any fees associated with this response to Deposit Account No. 13-3402.

Respectfully submitted,



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Attorney Docket No.: **WEICKM-0014**

Date: July 7, 2006

1: J Bacteriol. 2000 Sep;182(17):4941-50.

Expression of a constitutively active Cdc42 homologue promotes development of sclerotic bodies but represses hyphal growth in the zoopathogenic fungus Wangiella (Exophiala) dermatitidis.

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In contrast to the CDC42 homologues of Saccharomyces cerevisiae and Schizosaccharomyces pombe, the WdCDC42 gene in the human pathogenic fungus Wangiella (Exophiala) dermatitidis was found to be nonessential for cell viability. Expression of the constitutively active allele wdcdc42(G14V) at 37 degrees C induced nonpolarized growth that led to cell enlargement and multiple nucleation. The swollen cells subsequently converted into planate divided bicellular forms or multiply septated sclerotic bodies in post-log phase, when the G14V-altered protein was diminished. The wdcdc42(G14V) mutation also strongly repressed filamentous growth both in the wild-type strain and in the temperature-sensitive hyphal-form mutant Hf1. In contrast, overexpression of the dominant negative alleles wdcdc42(T19N) and wdcdc42(D120A) had no obvious effect on fungal-cell polarization. These results suggested that WdCdc42p plays a unique regulatory role in cellular morphogenesis in W. dermatitidis. Activation of this protein in response to extracellular or intracellular signals seems to commit its yeast-like cells to a phenotype transition that produces sclerotic bodies while repressing hyphal development.

PMID: 10940039 [PubMed - indexed for MEDLINE]

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Services as First Class Mail in an envelope addressed to: Commissioner of Patents, P O Box 1450, Alexandria, VA 22313-1450 on: July 7, 2006
Name: Heidi Wallenhorst
Signature: Heidi Wallenhorst

1: Cell Cycle. 2005 Nov;4(11):1675-82. Epub 2005 Nov 13.

Related Articles, Links

LANDES BIOSCIENCE

Constitutively active Cdc42 mutant confers growth disadvantage in cell transformation.

Vanni C, Ottaviano C, Guo F, Puppo M, Varesio L, Zheng Y, Eva A.

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The Rho family small GTPase Cdc42 is critical for diverse cellular functions including the regulation of actin organization, cell polarity, intracellular membrane trafficking, transcription, cell cycle progression and cell transformation. Like other members of the Rho family, Cdc42 cycles between the GTP-bound, active state, and the inactive, GDP-bound state under tight regulation, and it is believed that the GTP bound form of Cdc42 represents the active signaling module in eliciting effector activation and cellular responses. The constitutively active mutant, V12, derived from the analogous mutations found in oncogenic Ras that are GTPase-defective, and a "fast-cycling" self-activating mutant, F28, of Cdc42, have been widely in use to study the cellular effects of Cdc42. Here we report that the constitutively active V12 mutant of Cdc42, when stably expressed in cells, could behave in a dominant negative fashion in inhibiting cell proliferation while the F28 mutant was growth stimulatory. The V12 mutant failed to transform NIH3T3 cells while F28 potently stimulated anchorage-independent growth. The growth inhibitory effect of the V12 mutant correlated with activation of JNK2 and suppression of the cyclin D1 and NF-kappaB expressions that were instead upregulated by the F28 mutant. Furthermore, the V12 mutant could suppress, whereas the F28 mutant potentiated or had no effect on, a wide variety of oncogene-induced cell transformation, including that by the Dbl family GEFs Dbl, Vav and Lbc and the oncogenic Ras, ErbB-2, PDGF B or Raf. These results raise the possibility that over-saturation or constitutive activation of Cdc42 signal may negatively impact on cell proliferation and that both the activation and deactivation steps, or the complete GTPase cycle, of Cdc42 is required for proper function.

PMID: 16294011 [PubMed - in process]

1: Mol Cell Biol. 1996 Apr;16(4):1595-603.

Activation and association of Stat3 with Src in v-Src-transformed cell lines.

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STAT proteins are a group of latent cytoplasmic transcription factors which function as signal transducers and activators of transcription. Stat1 and -2 were originally identified to function in interferon signaling, and Stat1 was also found to be activated by epidermal growth factor (EGF) and other cytokines. New members of the STAT gene family are identified. Among them, Stat3 has 52.5% amino acid sequence homology with Stat1 and is activated by platelet-derived growth factor (PDGF), colony-stimulating factor 1 (CSF-1), EGF, interleukin-6, and other cytokines. Treatment of cells with EGF activates Stat1 and Stat3, which become phosphorylated on tyrosine residues to form homo - or heterodimers and translocate into the nucleus, binding to the sis-inducible element (SIE) in the c-fos promoter. Somatic cell genetic analyses demonstrated that Jaks, a family of nontransmembrane protein tyrosine kinases, are required for the activation of Stat1 and Stat2 in interferon-treated cells. However, little is known about the activation of Stat3 by growth factors. Here we report that in all v-Src-transformed cell lines examined, Stat3 is constitutively activated to bind to DNA and the phosphorylation of tyrosine on Stat3 is enhanced by the induction of v-Src expression. We also report that Src is shown to be associated with Stat3 in vivo, as well as in vitro, and phosphorylates Stat3 in vitro. Stat3 is also activated by CSF-1, possibly through CSF-1 receptor-c Src association in NIH 3T3 cells overexpressing CSF-1 receptors. Together, the data suggest that Src is involved in activation of Stat3 in growth factor signal transduction.

PMID: 8657134 [PubMed - indexed for MEDLINE]

1: Blood. 1999 Oct 1;94(7):2433-44.

Related Articles, Links

M-Ras, a widely expressed 29-kD homologue of p21 Ras: expression of a constitutively active mutant results in factor-independent growth of an interleukin-3-dependent cell line.

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M-Ras, a recently identified homologue of p21 Ras, is widely expressed, with levels of the 29-kD protein in spleen, thymus, and NIH 3T3 fibroblasts equaling or exceeding those of p21 Ras. A G22V mutant of M-Ras was constitutively active and its expression in an interleukin-3 (IL-3)-dependent mast cell/megakaryocyte cell line resulted in increased survival in the absence of IL-3, increased growth in IL-4, and, at high expression levels, in factor-independent growth. Expression of M-Ras G22V, however, had a negative effect on growth in the presence of IL-3, suggesting that M-Ras has both positive and negative effects on growth. Expression of M-Ras G22V in NIH-3T3 fibroblasts resulted in morphological transformation and growth to higher cell densities. M-Ras G22V induced activation of the c-fos promoter, and bound weakly to the Ras-binding domains of Raf-1 and RalGDS. Expression of a mutant of M-Ras G22V that was no longer membrane-bound partially inhibited (40%) activation of the c-fos promoter by N-Ras Q61K, suggesting that M-Ras shared some, but not all, of the effectors of N-Ras. An S27N mutant of M-Ras, like the analogous H-Ras S17N mutant, was a dominant inhibitor of activation of the c-fos promoter by constitutively active Src Y527F, suggesting that M-Ras and p21 Ras shared guanine nucleotide exchange factors and are likely to be activated in parallel. Moreover, M-Ras was recognized by the monoclonal anti-Ras antibody Y13-259, commonly used to study the function and activity of p21 Ras. Mammalian M-Ras and a Caenorhabditis elegans orthologue exhibit conserved structural features, and these are likely to mediate activation of distinctive signaling paths that function in parallel to those downstream of p21 Ras.

PMID: 10498616 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 2003 Dec 26;278(52):52154-65. Epub 2003 Oct 9.

Constitutively active Galpha16 stimulates STAT3 via a c-Src/JAK- and ERK-dependent mechanism.

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The hematopoietic-specific Galpha16 protein has recently been shown to mediate receptor-induced activation of the signal transducer and activator of transcription 3 (STAT3). In the present study, we have delineated the mechanism by which Galpha16 stimulates STAT3 in human embryonic kidney 293 cells. A constitutively active Galpha16 mutant, Galpha16QL, stimulated STAT3-dependent luciferase activity as well as the phosphorylation of STAT3 at both Tyr705 and Ser727. Galpha16QL-induced STAT3 activation was enhanced by overexpression of extracellular signal-regulated kinase 1 (ERK1), but was inhibited by U0126, a Raf-1 inhibitor, and coexpression of the dominant negative mutants of Ras and Rac1. Inhibition of phospholipase Cbeta, protein kinase C, and calmodulin-dependent kinase II by their respective inhibitors also suppressed Galpha16QL-induced STAT3 activation. The involvement of tyrosine kinases such as c-Src and Janus kinase 2 and 3 (JAK2 and JAK3) in Galpha16QL-induced activation of STAT3 was illustrated by the combined use of selective inhibitors and dominant negative mutants. In contrast, c-Jun N-terminal kinase, p38 MAPK, RhoA, Cdc42, phosphatidylinositol 3-kinase, and the epidermal growth factor receptor did not appear to be required. Similar observations were obtained with human erythroleukemia cells, where STAT3 phosphorylation was stimulated by C5a in a PTX-insensitive manner. Collectively, these results highlight the important regulatory roles of the Ras/Raf/MEK/ERK and c-Src/JAK pathways on the stimulation of STAT3 by activated Galpha16. Demonstration of the involvement of different kinases in Galpha16QL-induced STAT3 activation supports the involvement of multiple signaling pathways in the regulation of transcription by G proteins.

PMID: 14551213 [PubMed - indexed for MEDLINE]